

PAPER • OPEN ACCESS

The effect of L-rhamnose on intestinal transit time, short chain fatty acids and appetite regulation: a pilot human study using combined $^{13}\text{CO}_2/\text{H}_2$ breath tests

To cite this article: Claire S Byrne *et al* 2018 *J. Breath Res.* **12** 046006

View the [article online](#) for updates and enhancements.

Related content

- [Simultaneous non-invasive measurement of liquid gastric emptying and small bowel transit by combined \$^{13}\text{C}\$ -acetate and \$\text{H}_2\$ -lactulose breath test](#)
Franziska Bertram, Viola Andresen, Peter Layer *et al.*
- [Characterization of delayed liquid gastric emptying in children by the \$^{13}\text{C}\$ -acetate breath test](#)
B L Jones, R E Pearce, S M Abdel-Rahman *et al.*
- [Topical Review](#)
Anil S Modak

**BREATH[®]
BIOPSY**

**CONFERENCE
2018**

Breath Biopsy Conference

 **8TH NOVEMBER**  **CAMBRIDGE, UK**

[LEARN MORE](#)

Journal of Breath Research



PAPER

OPEN ACCESS

RECEIVED
27 April 2018REVISED
9 July 2018ACCEPTED FOR PUBLICATION
17 July 2018PUBLISHED
6 August 2018

Original content from this work may be used under the terms of the [Creative Commons Attribution 3.0 licence](#).

Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI.



The effect of L-rhamnose on intestinal transit time, short chain fatty acids and appetite regulation: a pilot human study using combined $^{13}\text{C}_2/\text{H}_2$ breath tests

Claire S Byrne¹ , Tom Preston², Jerusa Brignardello³, Isabel Garcia-Perez³, Elaine Holmes³, Gary S Frost¹ and Douglas J Morrison²

¹ Nutrition and Dietetic Research Group, Division of Diabetes, Endocrinology and Metabolism, Faculty of Medicine, Imperial College London, Hammersmith Hospital, London, United Kingdom

² Stable Isotope Biochemistry Laboratory, Scottish Universities Environmental Research Centre, University of Glasgow, East Kilbride, Glasgow, United Kingdom

³ Department of Surgery and Cancer, Computational and Systems Medicine, Imperial College London, South Kensington Campus, London, United Kingdom

E-mail: Douglas.Morrison@glasgow.ac.uk

Keywords: dietary fibre, short chain fatty acids, propionate, gastric emptying, oro-caecal transit time, ^{13}C breath test, peptide YY

Supplementary material for this article is available [online](#)

Abstract

Background. The appetite-regulating effects of non-digestible carbohydrates (NDC) have in part previously been attributed to their effects on intestinal transit rates as well as microbial production of short chain fatty acids (SCFA). Increased colonic production of the SCFA propionate has been shown to reduce energy intake and stimulate gut hormone secretion acutely in humans. **Objective.** We investigated the effect of the propiogenic NDC, L-rhamnose, on gastrointestinal transit times using a combined $^{13}\text{C}_2/\text{H}_2$ breath test. We hypothesised that L-rhamnose would increase plasma propionate leading to a reduction in appetite, independent of changes in gastrointestinal transit times. **Design.** We used a dual ^{13}C -octanoic acid/lactose ^{13}C -ureide breath test combined with breath H_2 to measure intestinal transit times following the consumption of 25 g d^{-1} L-rhamnose, compared with inulin and cellulose, in 10 healthy humans in a randomised cross-over design pilot study. Gastric emptying (GE) and oro-caecal transit times (OCTTs) were derived from the breath ^{13}C data and compared with breath H_2 . Plasma SCFA and peptide YY (PYY) were also measured alongside subjective measures of appetite. **Results.** L-rhamnose significantly slowed GE rates (by 19.5 min) but there was no difference in OCTT between treatments. However, breath H_2 indicated fermentation of L-rhamnose before it reached the caecum. OCTT was highly correlated with breath H_2 for inulin but not for L-rhamnose or cellulose. L-rhamnose consumption significantly increased plasma propionate and PYY but did not significantly reduce subjective appetite measures. **Conclusions.** The NDCs tested had a minimal effect on intestinal transit time. Our data suggest that L-rhamnose is partially fermented in the small intestine and that breath H_2 reflects the site of gastrointestinal fermentation and is only a reliable marker of OCTT for certain NDCs (e.g. inulin). Future studies should focus on investigating the appetite-suppressing potential of L-rhamnose and verifying the findings in a larger cohort.

Abbreviation list

ANOVA	analysis of variance	EEC	enteroendocrine cells
AOC	area over the curve	FFAR	free fatty acid receptor
AUC	area under the curve	GE	gastric emptying
CAS	composite appetite score	GLP-1	glucagon-like peptide-1
CRF	clinical research facility	IRMS	isotope ratio mass spectrometry

IQR	interquartile range
NDC	non-digestible carbohydrates
NIHR	National Institute of Health Research
OCTT	oro-caecal transit time
PDR	percentage dose recovery
PYY	peptide YY
RCF	relative centrifugal force
REC	research ethics committee
Rms	root mean square
SCFA	short chain fatty acids
SEM	standard error of the mean
VAS	visual analogue scale

Introduction

Epidemiological evidence suggests that non-digestible carbohydrates (NDC) play a key role in body weight management [1–3]. A number of underlying mechanisms have been suggested for this effect including the promotion of satiety, increased intraluminal viscosity, delayed absorption of nutrients and divergent effects on small and large intestinal transit times following NDC consumption [4]. In addition, it has been suggested that the production of short chain fatty acids (SCFA) via the microbial fermentation of NDC may play a role in mediating their appetite-suppressing effects. Increasing the colonic production of the SCFA propionate has been reported to significantly increase postprandial concentrations of the anorexigenic gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1), and to reduce *ad libitum* energy intake acutely in humans [5]. The identification of naturally propiogenic NDCs (i.e. NDC that lead to increased relative propionate production following their consumption) that successfully suppress appetite is therefore an attractive research avenue.

L-rhamnose, a non-absorbable monosaccharide, has previously been reported to be propiogenic [6, 7]. It is not actively absorbed in the small intestine and reaches the colon intact where it is fermented by a limited microbial community [8]. Inulin, a fructo-oligosaccharide, is an NDC that resists enzymatic digestion in the small intestine and is delivered to the large intestine intact [9]. Dietary supplementation with inulin has been shown to significantly increase caecal production of SCFA compared with cellulose in rodents [10] and been associated with a reduction in energy intake in humans, albeit at very high doses ($>35 \text{ g d}^{-1}$) [11]. It is however currently unknown how L-rhamnose compares to inulin in terms of its propionate-producing and appetite-suppressing capacity. In contrast, cellulose is often used as a reference fibre due

to its low and incomplete fermentability by the human gut microbiota [12]. We were interested to dissect the role of these NDCs on intestinal transit and SCFA production in relation to appetite regulation.

In the present study, we examined whether alterations in intestinal transit time or SCFA production profiles were associated with measures of appetite in healthy humans. We used a dual ^{13}C -octanoic acid/lactose ureide breath test [13, 14], both of which have been validated against scintigraphy [13, 15], combined with breath H_2 , which is routinely used as a measure of OCTT for some NDCs [14], to measure GE and OCTT rates following the consumption of L-rhamnose. We hypothesised that L-rhamnose would significantly increase circulating propionate concentrations and would have a similar intestinal transit rate compared with inulin (positive control) and cellulose (negative control).

Subjects and methods

This study was carried out in accordance with the Declaration of Helsinki and was approved by the London-Harrow Research Ethics Committee (REC) (REC Reference Number 14/LO/0704). All subjects provided informed, written consent.

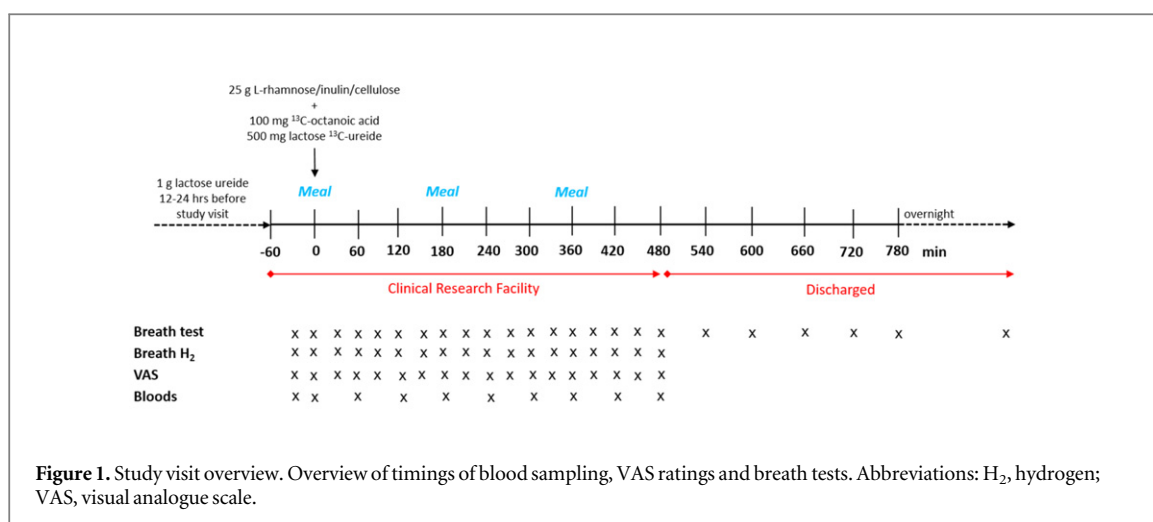
This pilot study was a randomised, double-blind, cross-over trial involving three study visits. The primary outcome measure was intestinal transit time and secondary outcome measures were plasma SCFA and PYY profiles and finally appetite scores. This was a pilot study because prior data on the effects of L-rhamnose on intestinal transit times was not available to undertake a sample-size calculation.

Participant recruitment

Participants were recruited via existing volunteer databases and poster advertisements. Healthy men and women aged between 18 and 65 years with BMI (kg m^{-2}) 20–35 were eligible for inclusion. Exclusion criteria included the following: weight gain or loss $\geq 5\%$ body weight in the preceding two months, current smokers, substance abuse, excess alcohol intake, any chronic illness or gastrointestinal disorder, pregnancy, or use of antibiotics and medications likely to interfere with energy metabolism. Eligible participants were randomised into the study by the sealed envelope randomisation service (www.sealedenvelope.com).

Dietary supplements

L-rhamnose (Carbosynth Limited, Compton, UK) was the interventional supplement used in this study. A dose of 25 g d^{-1} was chosen as L-rhamnose has previously been shown to increase serum propionate at this dosage [6, 7]. Inulin (Beneo HP, DKSH, London, UK) was used as a positive control (25 g d^{-1}). Inulin contains a range of fructose polymers with an



average chain length of 23. Cellulose (ACI Group Ltd, Slough, UK) was used as a negative control (25 g d^{-1}). Lactose- ^{13}C ureide and unlabelled lactose ureide were synthesised at the University of Glasgow as previously described [16].

Study day protocol

Participants were asked to avoid caffeine, alcohol and strenuous exercise, and to record their food intake for the 24 h before each study visit. Participants consumed the same evening meal before each visit in order to promote consistency between visits. Participants were also asked to consume 1 g unlabelled lactose ureide 12–24 h before their visit. This induced microbial activity to rapidly hydrolyse lactose- ^{13}C ureide, leading to a more definitive rise in breath $^{13}\text{CO}_2$ from fermentation [14]. Finally, participants were asked to arrive having fasted for 12 h prior to their study visit.

Study visits were conducted between January and May 2015 in the National Institute for Health Research (NIHR)/Wellcome Trust Imperial Clinical Research Facility (CRF), Hammersmith Hospital, London, United Kingdom. All participants attended three separate study visits ≥ 7 days apart. An overview of the study visit protocol is given in figure 1.

Prior to the consumption of the test meal (0 min), body composition was assessed by bioelectrical impedance analysis (Tanita BC-418 analyser; Tanita Corporation, Japan). Participants were asked to empty their bladders and change into a set of hospital scrubs prior to the collection of body composition data, in order to promote consistency between visits. In addition, a number of baseline samples were collected in duplicate (>10 min apart) prior to breakfast. Baseline blood samples were collected via a peripheral cannula in order to assess circulating SCFA concentrations. Baseline breath H_2 measurements were collected in real-time using a handheld breath H_2 metre (EC60 Gastrolyser Breath Hydrogen Monitor; Bedfont Scientific). Baseline breath samples were collected in order to measure $^{13}\text{CO}_2$ natural abundance and involved

blowing through a straw into an Exetainer (Labco Co., High Wycombe, UK) until vapour condensed at the bottom of the tube. Baseline subjective feelings of appetite ('how hungry/full do you feel?') and mood ('how nauseous do you feel?') were also collected using a series of 100 mm VAS. The left extremity of the visual analogue scale (VAS) was labelled with 'not at all' and the right-hand extremity was labelled with 'extremely'. Participants were asked to draw a vertical line on the VAS depending on how intensely they were experiencing each feeling.

Participants received a standardised breakfast (~ 470 kcal; supplementary table 1 is available online at stacks.iop.org/JBR/12/046006/mmedia) at 0 min consisting of 100 mg (1- ^{13}C)-Octanoic acid (Sercon Ltd, Crew, UK) and 500 mg lactose- ^{13}C ureide and 25 g L-rhamnose, inulin or cellulose, according to the participant's randomisation. Participants were provided with a standardised snack (~ 650 kcal) at 180 min and a standardised meal (~ 550 kcal) at 360 min (supplementary table 1). Further venous blood and breath samples were collected and VAS were completed throughout the study visit (figure 1). After 480 min, the cannula was removed and participants were discharged from the CRF.

Finally, participants were asked to collect breath samples hourly for 4 h following discharge and to collect a breath sample as soon as they woke up on the morning after their study visit in order to keep tracking breath $^{13}\text{CO}_2$ enrichment. In addition to this, participants were asked to consume the same evening meal after each visit to promote consistency between visits.

Blood sample preparation

5 ml venous blood samples were collected in plain 5 ml glass tubes and kept on ice until centrifugation. Samples were centrifuged at 2500 relative centrifugal force at 4°C for 10 min. Resulting plasma was aliquoted into cryovials and immediately frozen at -80°C until assaying for SCFA and PYY.

Breath sample analysis

Breath samples were analysed by isotope ratio mass spectrometry [17], a method used for measuring the relative abundance of isotopes in any given sample, as previously described [14].

Curve-fitting of breath $^{13}\text{CO}_2$ data

The fraction of tracer recovered in breath per hour (PDR h^{-1}) was calculated using Microsoft Excel software using the following equation:

$$\text{PDR h}^{-1} = [\text{VCO}_2, \text{breath } ^{13}\text{C enrichment} / \left(\frac{\text{mg dose}}{\text{MW}} \right) \cdot \text{dose enrichment} \cdot n] \cdot 100,$$

where VCO_2 is the CO_2 production rate (estimated to be $300 \text{ mmol m}^{-2} \text{ body surface h}^{-1}$; [18], with body surface estimated according to Haycock *et al* [19]), breath ^{13}C enrichment is the ^{13}C enrichment of breath over baseline (ppm excess), mg dose is the amount of substrate administered (mg), MW is the molecular weight of substrate, dose enrichment is the ^{13}C enrichment of substrate (ppm ^{13}C), and n is the number of isotopically labelled atoms in the substrate molecule.

Breath data were analysed using a modified version of the curve-fitting techniques developed by Ghooos *et al* [13] and Maes *et al* [20], as previously published [14]

$$Y = a_1(t-d_1)^{b_1} \cdot \exp(-c_1(t-d_1)) + a_2(t-d_2)^{b_2} \cdot \exp(-c_2(t-d_2)).$$

The variables a_i , b_i , c_i and d_i each describe a facet of each peak (i_1 , i_2) in the breath test data; a is the scaling factor related to the amplitude of the peak, b relates to the ascending slope, c relates to the downslope of the peak and d was introduced to facilitate the fitting of multiple peaks, as previously described [14]. The function was fitted to the data using Microsoft Excel

time from substrate ingestion to the time of maximal breath enrichment. Both $t_{1/2}$ and t_{max} were calculated as previously described [20] with the minor modification of adding the value of d_1 to each value to account for any delay in GE. The 'd' term for the OCTT curve, d_2 , was calculated as a measure of OCTT. It was defined as the time at which breath $^{13}\text{CO}_2$ excretion, from the modelled data (peak 2) was non-zero and was arbitrarily set at 0.01—the time when breath $^{13}\text{CO}_2$ excretion reached 0.01 PDR h^{-1} . This was undertaken using the GOALSEEK function in Excel to return the time (t) when the equation describing peak 2 (using the best-fit parameters) was equal to 0.01.

Breath H_2 excretion times

The first sustained rise in breath H_2 was determined for each volunteer on each visit and was considered a marker of OCTT.

Metabolic and hormone analysis

Thawed samples were vortexed and aliquoted into fresh cryotubes containing heparin sodium ($2 \mu\text{l}/100 \mu\text{l}$ sample; 1000 IU ml^{-1} , Wockhardt UK Ltd). Plasma PYY was measured using a previously established in-house specific and sensitive radioimmunoassay [22, 23]. Plasma SCFA were measured using an Agilent 7000C Triple Quadrupole GC/MS System according to a previously published method [24]. Data processing was performed using Agilent MassHunter Quantitative Analysis software and SCFA concentrations were integrated from a freshly prepared calibration curve.

Composite appetite score

A composite appetite score (CAS) was calculated from the VAS as previously described using the following formula [25]:

$$\text{CAS} = \frac{[\text{Hunger} + (100 - \text{Fullness}) + \text{Desire to Eat} + \text{Appetite for Meal}]}{4}.$$

software. The nonlinear regression Solver function was used to minimise the difference (root mean square) between the fitted curve and the raw data by adjusting the variables a , b , c and d for both curves simultaneously [21].

Two parameters defined from the fitted curve were used to describe the gastric emptying (GE) rate; $t_{1/2}$ and t_{max} . Both have previously been shown to significantly correlate with scintigraphy data, supporting their use as measures of GE [20]. $t_{1/2}$ is defined as the timepoint at which 50% of exhaled $^{13}\text{CO}_2$ is recovered under the GE curve. t_{max} is defined as the

Statistics

Data were checked for normality (defined as $P \geq 0.01$) using the Shapiro–Wilk test. Results are presented as means \pm standard error of the mean (SEM) or medians (interquartile range) if non-parametric. Missing VAS, breath H_2 and PYY data points were replaced with the mean group value at that timepoint. The average of two separate baseline measurements for breath H_2 , CAS, plasma SCFA and plasma PYY data, which were collected before breakfast, were used for the 0 min measurement. Baseline values were compared using a one-way analysis of variance (ANOVA).

Table 1. Participant characteristics.

Variable	Participants
Male $n(\%)$	4 (40%)
European Caucasian $n(\%)$	10 (100%)
Age (y)	58.9 ± 1.9
Weight (kg)	70.9 ± 1.9
BMI (kg m^{-2})	24.8 ± 0.7
Body fat (%)	28.2 ± 2.6

If baseline values were significantly different between groups, results are presented as percentage change from baseline. Area under the curve (AUC) and area over the curve (AOC) were calculated using the trapezoidal rule. AUC/AOC and breath data were compared using a one-way repeated measures ANOVA with post-hoc Fischer LSD test. If non-parametric, data were compared using a Friedman's test with post-hoc Wilcoxon signed rank tests. Post-prandial SCFA and PYY concentrations were compared with baseline concentrations using paired t-tests or Wilcoxon's signed rank tests. R^2 was calculated using linear regression. Significance was considered $P < 0.05$. Statistical analysis was performed using IBM SPSS Statistics v23–v24. Graphs were prepared using GraphPad Prism v5.0–7.0.

Results

Participants

Participant characteristics are given in table 1. Ten healthy men and women participated in this study.

Breath ^{13}C enrichment profiles and intestinal transit times

The profiles of breath $^{13}\text{CO}_2$ recovery and breath H_2 concentrations following the dual ^{13}C breath test and the consumption of cellulose, inulin and L-rhamnose are shown in figure 2.

A two-peak model was fitted to the data and used to determine GE and OCTT; individual data and group means are given in figure 3 and table 2, respectively.

There was a small but significant difference for GE rate ($t_{1/2}$) between groups ($P = 0.020$; table 2). Post-hoc tests revealed that the $t_{1/2}$ was longer for L-rhamnose compared with cellulose (effect size: mean \pm SEM; 0.35 ± 0.13 h, $P = 0.023$) and inulin (0.30 ± 0.11 h, $P = 0.023$), which suggests that L-rhamnose delayed GE by 19.5 min on average. Similarly, there was a significant difference in the t_{max} parameter for GE rate between groups ($P = 0.017$). Post-hoc analysis revealed that the t_{max} for L-rhamnose was longer compared with cellulose (0.45 ± 0.16 h, $P = 0.018$) and inulin (0.34 ± 0.11 h, $P = 0.012$), which also supports the observation that L-rhamnose delayed GE versus cellulose and inulin.

There was no significant difference between groups for the ' d ' term parameter calculated to describe OCTT (d_2 , $P = 0.21$). However, there was a significant difference in H_2 excretion times between groups ($P < 0.001$). Post-hoc tests revealed there was a shorter time to sustained H_2 excretion time for L-rhamnose ($P = 0.004$) and inulin ($P = 0.005$) compared with cellulose, suggesting a shorter OCTT for L-rhamnose and inulin as determined by H_2 excretion. L-rhamnose also had a shorter time to sustained H_2 excretion time compared with inulin suggesting a shorter OCTT ($P = 0.005$).

There was no significant difference in $t_{1/2}$, t_{max} , OCTT (d_2) or breath H_2 excretion times between visits independent of the NDC consumed ($P = 0.34$ – $P = 0.90$).

Correlation between OCTT as assessed by $^{13}\text{CO}_2$ and H_2 excretion

The correlations between $^{13}\text{CO}_2$ and H_2 excretion times following consumption of cellulose, inulin or L-rhamnose are shown in figure 4. As previously described by Schneider *et al* [26], $^{13}\text{CO}_2$ from lactose- ^{13}C ureide fermentation significantly correlated with the H_2 excretion times following the consumption of inulin ($r^2 = 0.89$, $P < 0.001$; figure 4). However, for both cellulose and L-rhamnose $^{13}\text{CO}_2$ data there was no significant correlation with the H_2 excretion times ($P = 0.63$ and 0.77 , respectively).

Plasma SCFA

There was no difference in the baseline concentrations of any SCFA between groups ($P = 0.32$ – 0.60). There was a difference in the AUC_{0-480} for propionate between treatments ($P < 0.001$; figure 5(D)). Post-hoc analysis revealed that AUC_{0-480} for propionate was significantly greater for L-rhamnose than cellulose (effect size: $1.36 \pm 0.15 \mu\text{M} \times \text{min}$, $P < 0.001$) and inulin (effect size: $1.41 \pm 0.13 \mu\text{M} \times \text{min}$, $P < 0.001$) confirming increased propionate production from L-rhamnose. There was also a difference in the AUC_{0-480} for acetate between treatments ($P < 0.001$; figure 5(B)). Post-hoc analysis revealed that the AUC_{0-480} for acetate was significantly greater for L-rhamnose than cellulose (effect size: $20.39 \pm 3.73 \mu\text{M} \times \text{min}$, $P < 0.001$) and inulin (effect size: $9.95 \pm 3.69 \mu\text{M} \times \text{min}$, $P = 0.025$), suggesting increased acetate production from L-rhamnose. In addition, the AUC_{0-480} for acetate following consumption of inulin was significantly larger than cellulose (effect size: $10.44 \pm 2.36 \mu\text{M} \times \text{min}$, $P = 0.002$). There was no significant difference in the AUC_{0-480} for butyrate between treatments ($P = 0.59$; figure 5(F)).

Following the consumption of L-rhamnose, there was a trend for propionate concentrations (figure 5(C)) to be higher than baseline at 120 min (effect size: $0.67 \pm 0.30 \mu\text{M}$, $P = 0.052$) and concentrations were significantly higher than baseline at 240 (effect size: $2.41 \pm 0.42 \mu\text{M}$, $P < 0.001$) and 360 min (effect size: $0.99 \pm 0.36 \mu\text{M}$, $P = 0.023$). There was no

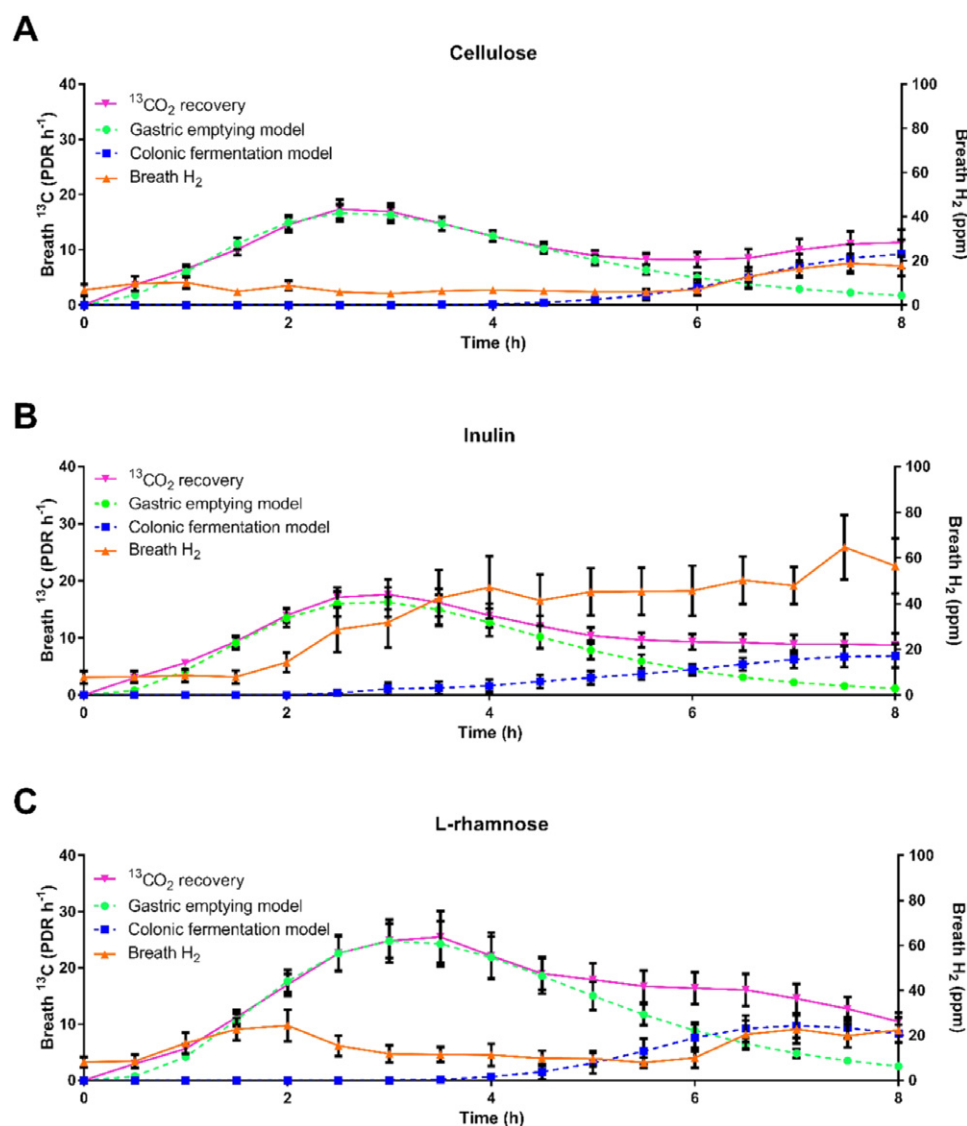


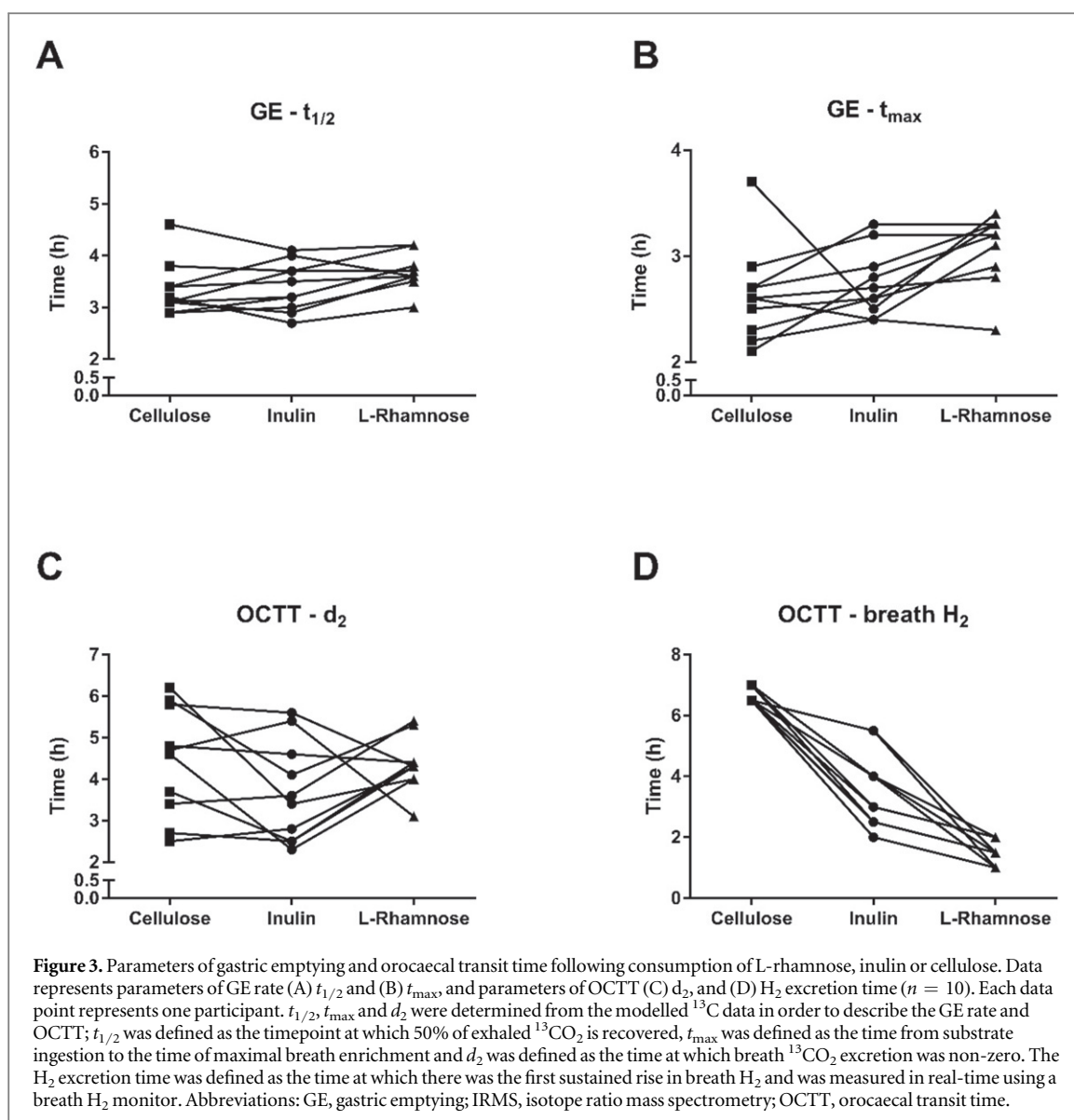
Figure 2. Breath H_2 concentrations and breath ^{13}C enrichment profiles following a dual ^{13}C breath test and the consumption of L-rhamnose, inulin or cellulose. Each graph displays mean \pm SEM (purple solid line) $^{13}CO_2$ recovery ($PDR\ h^{-1}$; left y axis), determined by IRMS analysis, (green dashed line) GE rates and (blue dashed line) OCTT ($PDR\ h^{-1}$; left axis), determined by a two-peak GE and colonic fermentation model, and (solid orange line) breath H_2 concentrations (ppm; right y axis) following the consumption of 100 mg ($1-^{13}C$)-Octanoic acid and 500 mg lactose- $[^{13}C]$ ureide and (A) cellulose, (B) inulin or (C) L-rhamnose at 0 min ($n = 10$). Abbreviations: GE, gastric emptying; IRMS, isotope ratio mass spectrometry; OCTT, oro-caecal transit time; PDR, percentage dose recovery.

difference in propionate concentrations at 480 min versus baseline (effect size: $0.75 \pm 0.56\ \mu M$, $P = 0.21$). Interestingly, despite the greater AUC_{0-480} following consumption of L-rhamnose versus cellulose and inulin, postprandial concentrations of acetate were not significantly higher than baseline at any timepoint measured ($P = 0.31-0.81$; figure 5(A)).

Plasma PYY

While the measurement of plasma PYY concentrations was not an *a priori* outcome measure, the significant increase in circulating propionate concentrations observed following the consumption of L-rhamnose prompted us to measure plasma PYY. There was no difference in the plasma PYY concentrations between

groups at baseline ($P = 0.47$; figure 6). There was a significant difference in the AUC_{0-420} for PYY between treatments ($P = 0.001$; figure 6(B)). Post-hoc analysis revealed that the AUC_{0-420} for PYY was significantly larger for L-rhamnose than cellulose (effect size: $9.77 \pm 4.0\ pmol\ l^{-1} \times min$, $P = 0.037$) and inulin (effect size: $16.41 \pm 4.16\ pmol\ l^{-1} \times min$, $P = 0.003$), suggesting higher concentrations following the consumption of L-rhamnose. PYY concentrations were significantly higher than baseline at all postprandial timepoints measured; 60 min (effect size: $14.11 \pm 4.88\ pmol\ l^{-1}$, $P = 0.018$), 180 min (effect size: $24.31 \pm 8.22\ pmol\ l^{-1}$, $P = 0.016$), 300 min (effect size: $31.04 \pm 7.32\ pmol\ l^{-1}$, $P = 0.002$) and 420 min (effect size: $28.38 \pm 9.70\ pmol\ l^{-1}$; $P = 0.017$).



Composite appetite score

There was a significant difference in CAS between groups at baseline ($P = 0.006$). This was due to participants reporting significantly lower CAS at baseline of the L-rhamnose visit compared with cellulose (effect size: 5.6 ± 2.3 mm, $P = 0.033$) and inulin (effect size: 9.7 ± 3.2 mm, $P = 0.015$). Therefore data are expressed as percentage change from baseline (figure 7). There was no difference between treatments for CAS area over the curve (AOC)₀₋₄₈₀ ($P = 0.37$).

Discussion

A dual ^{13}C breath test combined with breath H_2 excretion was used in this pilot study in order to evaluate effects on gut transit of three different NDC; cellulose, inulin and L-rhamnose. The aim of conducting this study was two-fold: (1) to ascertain if variations in transit time could explain an observed effect on appetite; (2) to examine the variability in the

optimum time-window for assessment of appetite where SCFA production from NDC is maximal and most likely to have an impact on satiety for future studies.

L-rhamnose significantly delayed GE when compared with cellulose and inulin ($t_{1/2}$: by 21 and 18 min, respectively). While the caloric load and nutritional composition of a meal are understood to affect GE rates [27], these factors are unlikely to have caused the differences observed in this study as the test meals were provided at exactly the same time in each group, were isoenergetic and had the same nutritional composition. Alternatively, the rate of GE has previously been shown to be strongly influenced by the osmolality of the contents of the duodenum, with increased osmolality slowing GE [28, 29]. Therefore, L-rhamnose may have resulted in a delay in GE versus inulin and cellulose due to its greater osmotic load. However, it is currently unknown whether the modest reduction in GE rate observed

Table 2. Parameters of gastric emptying and oro-caecal transit time following consumption of L-rhamnose, inulin or cellulose.

Breath test parameter	Cellulose (h)	Inulin (h)	L-rhamnose (h)	P-value ^a
GE rate				
$t_{1/2}$ ^b	3.4 ± 0.2 ^f	3.4 ± 0.1 ^f	3.7 ± 0.1 ^f	0.020
t_{\max} ^c	2.6 ± 0.1 ^f	2.7 ± 0.1 ^f	3.1 ± 0.1 ^f	0.017
OCTT				
d_2 ^d	4.4 ± 0.4	3.7 ± 0.4	4.4 ± 0.2	0.21
H ₂ excretion time ^e	6.5 [6.5, 7.0] ^f	3.5 [2.5, 4.0] ^f	1.5 [1.0, 2.0] ^f	<0.001

Note. Parametric data are expressed as means ± SEMs and non-parametric data are expressed as median [IQR] ($n = 10$). Units are in h. Abbreviations: GE, gastric emptying; NDC, non-digestible carbohydrate; OCTT, oro-caecal transit time.

^{b,c,d} Parameters were determined from the modelled ¹³C data in order to describe the GE rate and OCTT.

^a Parametric data were compared using a one-way repeated measures ANOVA with post-hoc Fischer LSD test. Non-parametric data were compared using a Friedman's test with post-hoc Wilcoxon Signed Ranks Test.

^b $t_{1/2}$ was defined as the timepoint at which 50% of exhaled ¹³CO₂ is recovered.

^c T_{\max} was defined as the time from substrate ingestion to the time of maximal breath enrichment and was calculated using the following equation $T_{\max} = (b/c) + d$.

^d d_2 , a measure of OCTT, was defined as the time at which breath ¹³CO₂ excretion, from the modelled data (peak 2) was non-zero and was arbitrarily set at 0.01—the time when breath ¹³CO₂ excretion reached 0.01 PDR h⁻¹. This was undertaken using the GOALSEEK function in Excel to return the time (t) when the equation describing peak 2 (and using the best-fit parameters) was equal to 0.01.

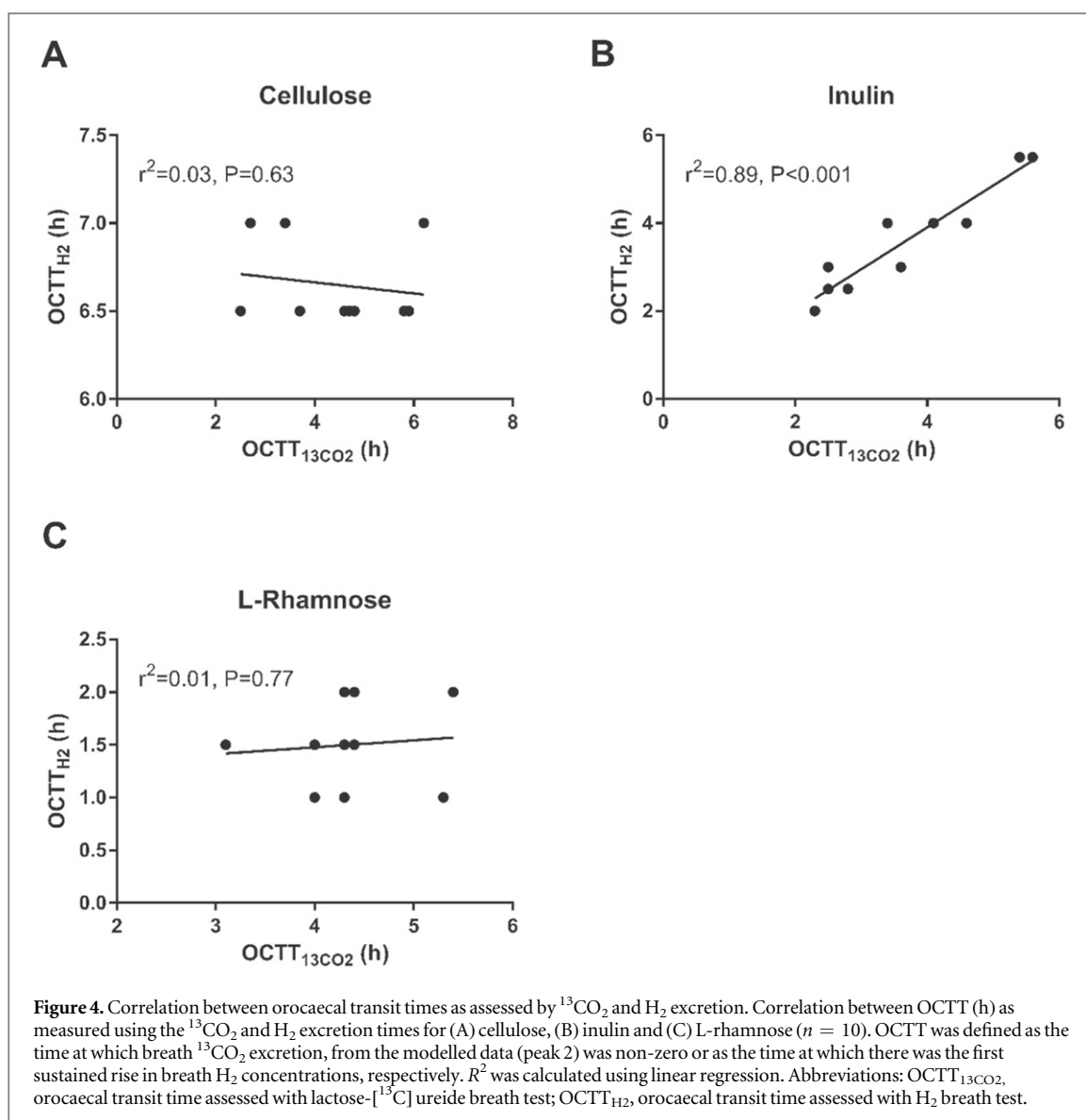
^e H₂ excretion time was defined as the time at which there was the first sustained rise in breath H₂ concentrations.

^f Indicate significant differences in post-hoc tests.

following the consumption of L-rhamnose would have a significant impact on eating behaviour.

Despite detecting differences in GE rates between the NDC there were no differences in OCTT as measured from breath ¹³C excretion. However, in contrast, breath H₂ excretion times suggest a significantly shorter transit time for L-rhamnose and inulin versus cellulose, and a significantly shorter transit time for L-rhamnose versus inulin. This difference in gut transit times as assessed using breath H₂ and ¹³CO₂ was an unexpected finding and not in line with the original hypothesis. The H₂-based inulin breath test has previously been used in multiple research studies as a reliable measure of OCTT and has been shown by Schneider *et al* to correlate well with the lactose-[¹³C] ureide breath test data following the consumption of inulin [26]. Similarly, in the present study, transit time determined from the breath H₂ excretion times significantly correlated with the ¹³C data following the consumption of inulin thus validating our data modelling approach and confirming fermentation of inulin in the caecum. However, for both cellulose and L-rhamnose the ¹³C data does not correlate with the H₂ excretion data. It is possible that differences in the sites of fermentation of these substrates may explain the findings. Despite the perception that L-rhamnose reaches the colon intact [7], the early rise in breath H₂ in the present study occurs before the stomach has fully emptied and before L-rhamnose has reached the caecum according to the ¹³C data suggesting that L-rhamnose is being partially fermented in the small

intestine. In support of this hypothesis, Boojink *et al* reported the presence of species related to *Ruminococcus obeum* in the ileal effluent of humans [30], which has previously been shown to ferment L-rhamnose via the propanediol pathway leading to the production of propionate [8]. In contrast to L-rhamnose and inulin, cellulose is thought to have low or incomplete fermentability [12]. However, in the present study there was a small but sustained rise in breath H₂ from 6 h following the consumption of cellulose, consistent with observations from previous studies [31], and suggests that cellulose is fermented more distally in the colon compared with inulin [32]. There was also a similar later rise in breath H₂ following consumption L-rhamnose, which corresponds with the ¹³C data and appears to represent fermentation of L-rhamnose in the colon. Together, the presented data suggest there was no difference in OCTT for the three NDCs tested but a difference in the fermentability and the site of fermentation within the intestine of the substrates. Furthermore, these data suggest that breath H₂ is only a suitable measure of OCTT following the consumption of certain NDCs (e.g. inulin) and its use as a marker of OCTT is unreliable following consumption of some NDCs (e.g. L-rhamnose and cellulose). Therefore, it is proposed that breath H₂ should only be used as a reliable marker of fermentability and not OCTT unless independent verification of the site of fermentation can be established.



Plasma propionate concentrations were significantly higher following consumption of L-rhamnose versus inulin and cellulose, which supports the hypothesis that L-rhamnose is propiogenic [6, 7]. Plasma propionate concentrations were elevated above baseline from 120 to 360 min ($P = 0.023$). This early increase in plasma propionate is observed following breath H_2 excretion and is detected in the peripheral circulation before L-rhamnose has reached the caecum according to the ^{13}C data. Together, these data further support the proposal that L-rhamnose does not reach the colon intact, as previously suggested, and is partially fermented in the small intestine. Interestingly, plasma acetate concentrations were higher following L-rhamnose compared with inulin, which was unexpected. One possible explanation for this increase in acetate could be due to differences in the rate of fermentation of the NDC owing to differences in chain length. For example, other short chain inulin molecules like fructo-oligosaccharides are more rapidly fermented versus long-chain inulin [33], which undergoes less rapid fermentation [9].

The co-expression of the SCFA receptors, free fatty acid receptor (FFAR) 2 and FFAR3, in PYY and GLP-1 secreting enteroendocrine cells has prompted suggestions that SCFA production following the consumption of NDC may stimulate the secretion of these anorexigenic gut hormones [5]. In acute studies, increased colonic propionate has previously been shown to increase plasma PYY and GLP-1 concentrations in humans [5]. Therefore, it was originally hypothesised that the consumption of L-rhamnose would reduce appetite via an increase in colonic propionate production, which would stimulate the secretion of anorexigenic gut hormones. Although our data suggests that the increase in plasma propionate concentrations following the consumption of L-rhamnose may be due to an increased production of propionate in the small intestine as well as in the colon, the propionate data prompted us to measure plasma PYY concentrations [34]. As hypothesised, the increase in peripheral propionate levels observed following consumption of L-rhamnose was associated with a

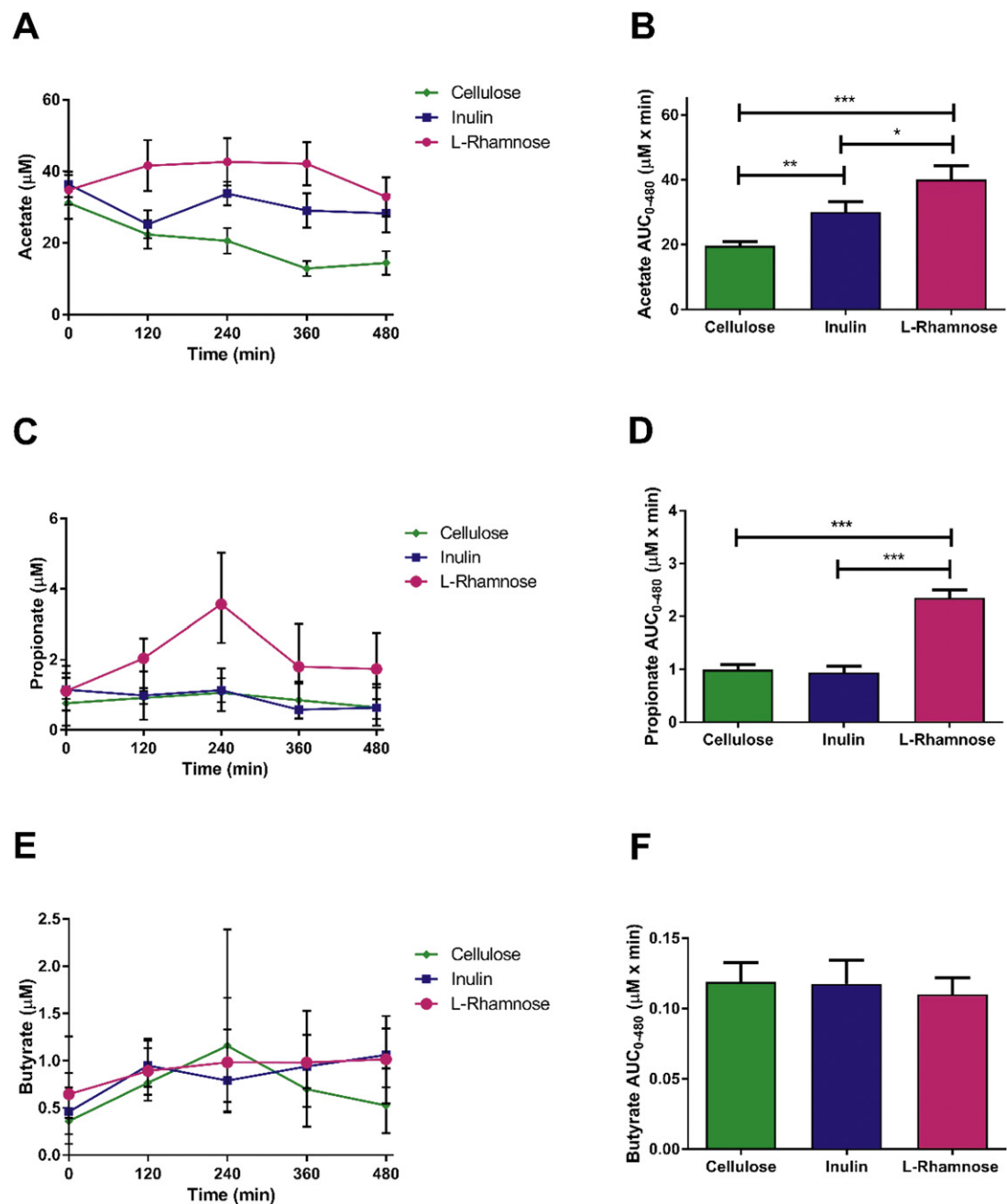
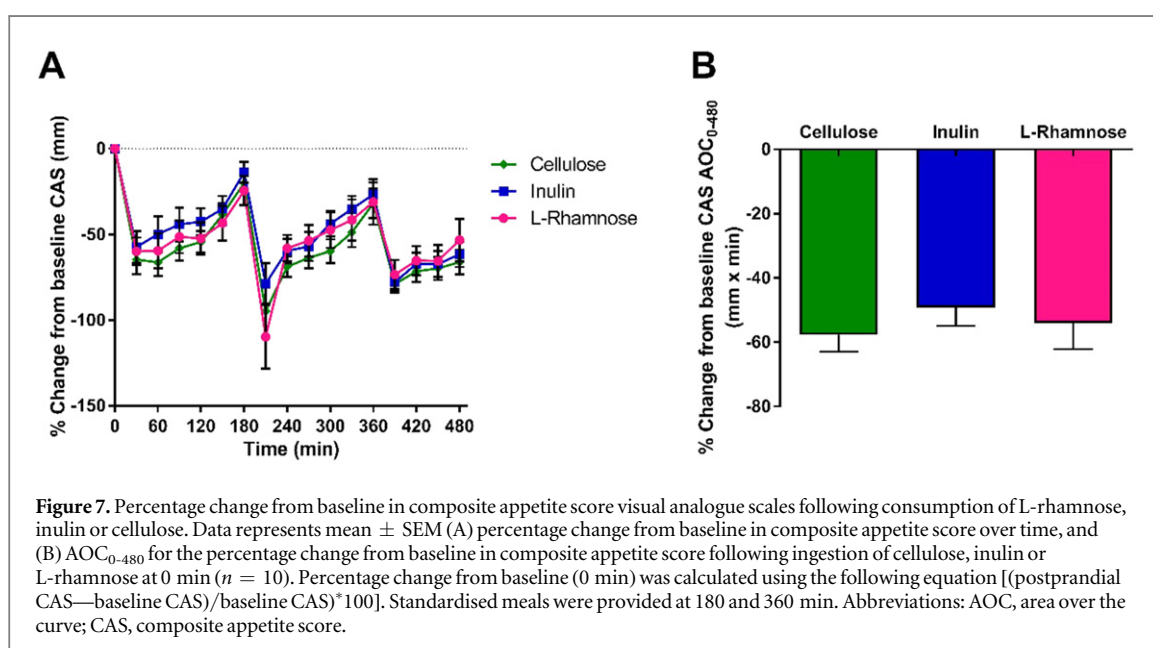
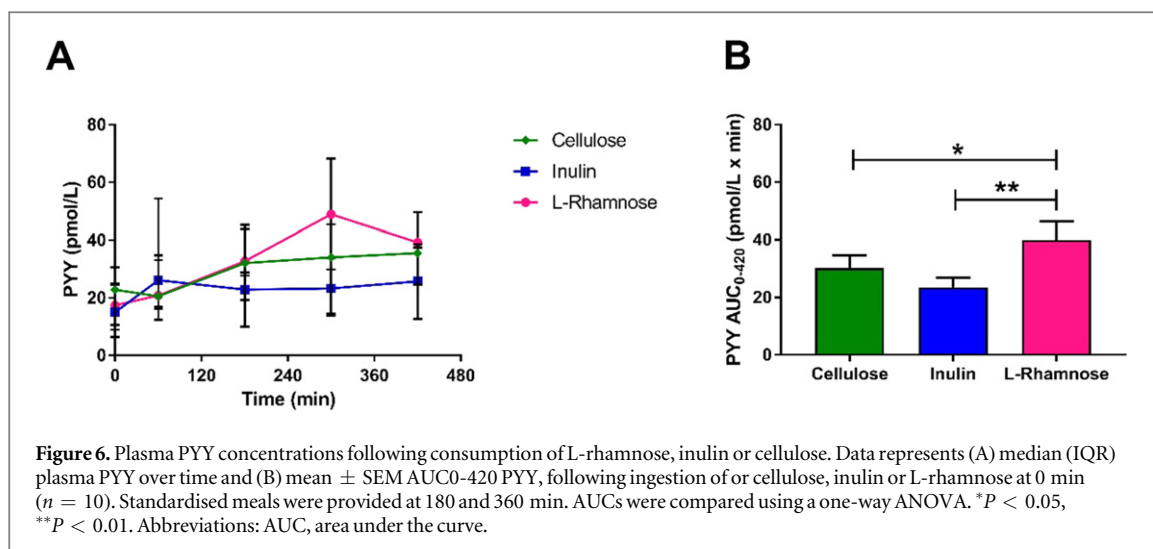


Figure 5. Plasma short chain fatty acid concentrations following consumption of L-rhamnose, inulin or cellulose. Data represents (A) mean \pm SEM acetate (C) median (IQR) propionate and (E) median (IQR) butyrate over time and mean \pm SEM (B) AUC₀₋₄₈₀ for acetate, (D) AUC₀₋₄₈₀ for propionate and (F) AUC₀₋₄₈₀ for butyrate following ingestion of cellulose, inulin or L-rhamnose at 0 min ($n = 10$). Standardised meals were provided at 180 and 360 min. AUCs were compared using a one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$. Abbreviations: AUC, area under the curve.

significant increase in plasma PYY concentrations versus inulin and cellulose and is the first time to our knowledge that L-rhamnose has been shown to increase plasma PYY concentrations. Interestingly, postprandial PYY was most elevated after L-rhamnose appeared to arrive in the colon. The increase in postprandial PYY concentrations noted in the current study suggests that L-rhamnose could influence satiety. However, as appetite was not the primary outcome of this pilot study, the only appetite measure collected were VAS. While there was no effect of treatment on the CAS, as this study was not adequately

powered to measure differences in VAS these data should be treated with caution. Interestingly, since the completion of the current study, Darzi *et al* reported that 25.5 g L-rhamnose does not reduce appetite [35]. However, *ad libitum* food intake was measured at 420 min, which is significantly later than the peak propionate and PYY concentrations noted in our study (240–300 min), suggesting effects on appetite may have been missed and should be addressed in future studies. Finally, a notable finding in the current study is the large inter- and intra-individual variability in GI transit times as outlined in figure 3, which others have



previously reported [36, 37]. While the data from the current study are in line with the literature, the variations in gut transit times further emphasise the challenges when designing such studies aiming to detect differences in appetite.

The use of a dual ^{13}C breath test, which has previously been validated against scintigraphy [13, 15], and a human cross-over study design are major strengths of this trial. However, the small sample-size in this pilot study is a limitation. Future studies are required in order to verify our findings in a larger cohort.

In summary, the presented data suggest that the NDC tested have minimal effects on gut transit time. Despite previously being considered to be fermented exclusively in the colon, the presented ^{13}C breath data, breath H_2 and plasma SCFA data suggest that L-rhamnose also undergoes microbial fermentation in the small intestine. Furthermore, our data suggests that breath H_2 should only be used as a reliable marker of fermentability and not OCTT unless corroborated

by an independent measure of OCTT. Finally, despite elevated propionate and PYY concentrations following consumption of L-rhamnose in addition to L-rhamnose slowing GE rate, L-rhamnose did not suppress subjective feelings of appetite. Further work is needed to assess whether these effects would alter eating behaviour.

Acknowledgments

The authors thank all the participants for their time and effort, as well as the University of Glasgow for supporting the analysis of the ^{13}C breath test samples. Our thanks also go to Mrs Sandra Small for collating the $^{13}\text{CO}_2$ data.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

CSB, GSF and DJM designed the research; CSB conducted the research; TP and DM provided essential materials; CSB, TP, JB, IGP, DM analysed the data; CSB, GSF and DJM interpreted the data; CSB, GSF and DJM wrote the paper; DM had primary responsibility for final content. All authors read and approved the final manuscript.

Disclaimers

The authors declare that there are no disclaimers.

Sources of support

This article presents independent research funded by Imperial College (IC) and supported by the NIHR CRF and BRC at IC Healthcare NHS Trust. The views expressed are those of the author(s) and not necessarily those of IC, the NHS, the NIHR or the Department of Health. The Division of Diabetes, Endocrinology and Metabolism and Investigative Medicine is funded by grants from the MRC, BBSRC, NIHR, an Integrative Mammalian Biology (IMB) Capacity Building Award, an FP7- HEALTH- 2009- 241592 EuroCHIP grant and is supported by the NIHR Biomedical Research Centre Funding Scheme. GF holds an NIHR Senior Investigator Award, CSB was funded by the United Kingdom Medical Research Council (MR/K01711X/1).

Clinical trial registry

This study was registered on the ISRCTN registry [https://www.isrctn.com/\(ISRCTN9022576\)](https://www.isrctn.com/(ISRCTN9022576)).

ORCID iDs

Claire S Byrne  <https://orcid.org/0000-0001-7578-6237>

Gary S Frost  <https://orcid.org/0000-0003-0529-6325>

Douglas J Morrison  <https://orcid.org/0000-0002-4161-5699>

References

- [1] Ludwig D S, Pereira M A, Kroenke C H, Hilner J E, Van Horn L, Slattery M L and Jacobs D R Jr 1999 Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults *J. Am. Med. Assoc.* **282** 1539–46
- [2] Du H, Boshuizen H C, Forouhi N G, Wareham N J, Halkjær J, Tjønneland A, Overvad K, Jakobsen M U, Boeing H and Buijsse B 2009 Dietary fiber and subsequent changes in body weight and waist circumference in European men and women *Am. J. Clin. Nutr.* **91** 329–36
- [3] Liu S, Willett W C, Manson J E, Hu F B, Rosner B and Colditz G 2003 Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women *Am. J. Clin. Nutr.* **78** 920–7
- [4] Müller M, Canfora E E and Blaak E E 2018 Gastrointestinal transit time, glucose homeostasis and metabolic health: modulation by dietary fibers *Nutrients* **10** 275
- [5] Chambers E S, Viardot A, Psichas A, Morrison D J, Murphy K G, Zac-Varghese S E, MacDougall K, Preston T, Tedford C and Finlayson G S 2014 Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults *Gut* **64** 1744–54
- [6] Vogt J A, Ishii-Schrade K B, Pencharz P B and Wolever T M 2004 L-Rhamnose increases serum propionate after long-term supplementation, but lactulose does not raise serum acetate *Am. J. Clin. Nutr.* **80** 1254–61
- [7] Vogt J A, Pencharz P B and Wolever T M 2004 L-rhamnose increases serum propionate in humans *Am. J. Clin. Nutr.* **80** 89–94
- [8] Reichardt N, Duncan S H, Young P, Belenguer A, McWilliam Leitch C, Scott K P, Flint H J and Louis P 2014 Phylogenetic distribution of three pathways for propionate production within the human gut microbiota *ISME J.* **8** 1323–35
- [9] Roberfroid M B, Van Loo J A and Gibson G R 1998 The bifidogenic nature of chicory inulin and its hydrolysis products *J. Nutr.* **128** 11–9
- [10] Weitkunat K, Schumann S, Petzke K J, Blaut M, Loh G and Klaus S 2015 Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice *J. Nutr. Biochem.* **26** 929–37
- [11] Pedersen C, Lefevre S, Peters V, Patterson M, Ghatei M A, Morgan L M and Frost G S 2013 Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. A dose-escalation study *Appetite* **66** 44–53
- [12] Topping D L and Clifton P M 2001 Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides *Physiol. Rev.* **81** 1031–64
- [13] Ghoo Y F, Maes B D, Geypens B J, Mys G, Hiele M I, Rutgeerts P J and Vantrappen G 1993 Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test *Gastroenterology* **104** 1640–7
- [14] Morrison D, Dodson B, Preston T and Weaver L 2003 Gastrointestinal handling of glycosyl [¹³C] ureides *Eur. J. Clin. Nutr.* **57** 1017–24
- [15] Geypens B, Bennink R, Peeters M and Evenepoel P 1999 Validation of the lactose-[¹³C] ureide breath test for determination of orocecal transit time by scintigraphy *J. Nucl. Med.* **40** 1451–4
- [16] Morrison D J, Dodson B, Preston T and Weaver L T 2001 Rapid quality control analysis of ¹³C-enriched substrate synthesis by isotope ratio mass spectrometry *Rapid Commun. Mass Spectrom.* **15** 1279–82
- [17] O'Hara J, Carroll S, Cooke C B, Morrison D J, Preston T and King R 2012 Preexercise galactose and glucose ingestion on fuel use during exercise *Med. Sci. Sports Exerc.* **44** 1958–67
- [18] Shreeve W, Cerasi E and Luft R 1970 Metabolism of [2-¹⁴C] pyruvate in normal, acromegalic and HGH-treated human subjects *Acta Endocrinol.* **65** 155–69
- [19] Haycock G B, Schwartz G J and Wisotsky D H 1978 Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults *J. Pediatr.* **93** 62–6
- [20] Maes B, Ghoo Y, Geypens B, Mys G, Hiele M, Rutgeerts P and Vantrappen G 1994 Combined carbon-13-glycine/carbon-14-octanoic acid breath test to monitor gastric emptying rates of liquids and solids *J. Nucl. Med.* **35** 824–31
- [21] Motulsky H J and Ransnas L A 1987 Fitting curves to data using nonlinear regression: a practical and nonmathematical review *FASEB J.* **1** 365–74
- [22] Adrian T, Ferri G, Bacarese-Hamilton A, Fuessl H, Polak J and Bloom S 1985 Human distribution and release of a putative new gut hormone, peptide YY *Gastroenterology* **89** 1070–7
- [23] Kreymann B, Ghatei M, Williams G and Bloom S 1987 Glucagon-like peptide-1 7-36: a physiological incretin in man *Lancet* **330** 1300–4

- [24] Moreau N, Gouptry S, Antignac J, Monteau F, Le Bizec B, Champ M, Martin L and Dumon H 2003 Simultaneous measurement of plasma concentrations and ^{13}C -enrichment of short-chain fatty acids, lactic acid and ketone bodies by gas chromatography coupled to mass spectrometry *J. Chromatogr. B* **784** 395–403
- [25] Woodend D M and Anderson G H 2001 Effect of sucrose and safflower oil preloads on short term appetite and food intake of young men *Appetite* **37** 185–95
- [26] Schneider A, Jepp K, Murczynski L, Biniek U and Stein J 2007 The inulin hydrogen breath test accurately reflects oro-caecal transit time *Eur. J. Clin. Invest.* **37** 802–7
- [27] Calbet J and MacLean D 1997 Role of caloric content on gastric emptying in humans *J. Physiol.* **498** 553–9
- [28] Hunt J 1960 The site of receptors slowing gastric emptying in response to starch in test meals *J. Physiol.* **154** 270–6
- [29] Meeroff J C, Go V L W and Phillips S F 1975 Control of gastric emptying by osmolality of duodenal contents in man *Gastroenterology* **68** 1144–51
- [30] Booiijink C C, El-Aidy S, Rajilić-Stojanović M, Heilig H G, Troost F J, Smidt H, Kleerebezem M, De Vos W M and Zoetendal E G 2010 High temporal and inter-individual variation detected in the human ileal microbiota *Environ. Microbiol.* **12** 3213–27
- [31] Oku T and Nakamura S 2014 Evaluation of the relative available energy of several dietary fiber preparations using breath hydrogen evolution in healthy humans *J. Nutr. Sci. Vitaminol.* **60** 246–54
- [32] Gray D, Eastwood M, Brydon W and Fry S 1993 Fermentation and subsequent disposition of ^{14}C -labelled plant cell wall material in the rat *Br. J. Nutr.* **69** 189–97
- [33] Stewart M L, Timm D A and Slavin J L 2008 Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an *in vitro* fermentation system *Nutr. Res.* **28** 329–34
- [34] Larraufie P, Martin-Gallausiaux C, Lapaque N, Dore J, Gribble F M, Reimann F and Blottiere H 2018 SCFAs strongly stimulate PYY production in human enteroendocrine cells *Sci. Rep.* **8** 74
- [35] Darzi J, Frost G S, Swann J R, Costabile A and Robertson M D 2016 l-rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake *Appetite* **98** 142–9
- [36] Degen L and Phillips S 1996 Variability of gastrointestinal transit in healthy women and men *Gut* **39** 299–305
- [37] Birkebaek N, Memmert K, Mortensen J, Dirksen H and Christensen M 1990 Fractional gastrointestinal transit time: intra- and interindividual variation *Nucl. Med. Commun.* **11** 247–52